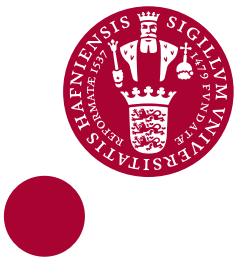

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Original Research Article

Response of primiparous and multiparous buffaloes to yeast culture supplementation during early and mid-lactation

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ABSTRACT

Strains of live *Saccharomyces cerevisiae* yeast have exhibited probiotic effects in ruminants. This study investigated the effects of the dietary yeast supplement, *S. cerevisiae* (Yea-Sacc^{1026}), on primiparous (PP) and multiparous (MP) Egyptian buffaloes in early to mid-lactation. Lactating buffaloes were fed either a basal total mixed ration (TMR, control; 4 PP and 8 MP) or the basal TMR plus 10 g Yea-Sacc^{1026} per buffalo cow per day (yeast; 4 PP and 8 MP). The feeds were given from 15 days prepartum to 180 days postpartum. Feed intake, body weight, and milk yields (MY) were recorded, and milk and blood samples were collected for analyses. Feces were collected from days 45 to 47 during early lactation and from days 90 to 92 during mid-lactation to determine apparent digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and crude fiber (CF). Energy corrected milk yield (ECM), feed conversion, and energy and nitrogen conversion efficiency were calculated. Yeast treated MP buffaloes consumed more DM ($P \leq 0.041$) and CP than the untreated control group. Apparent digestibility of DM and OM were significantly greater at mid-lactation for treated versus control group ($P = 0.001$). Crude fiber digestibility was greater in MP than in PP buffaloes ($P = 0.049$), and yeast supplemented MP cows had a greater CF digestibility than control MP buffaloes at mid-lactation ($P = 0.010$). Total blood lipids decreased after yeast supplementation ($P = 0.029$). Milk yields, ECM, fat and protein yields increased for yeast treated MP buffaloes ($P \leq 0.039$). The study concluded that the response to yeast supplementation in buffalo cows is parity dependent. Multiparous buffaloes respond to yeast supplementation with an increased DM intake and CF digestibility without significant weight gains, allowing a greater ECM yield with less fat mobilization. Supplementing buffaloes with yeast culture may increase milk production in early lactation and results in a more persistent milk production during mid-lactation. Feed conversion and energy and nitrogen conversion efficiency may be increased with the use of yeast supplementation in Egyptian buffaloes.

1. Introduction

High yielding dairy cows have difficulty in fully utilizing a nutritionally balanced ration because of physiological constraints in early lactation (McDonald et al., 2002). These problems may be overcome through yeast supplementation. It has been shown that yeast supplementation can increase conversion efficiency, stimulate rumen fiber digestion, stabilize ruminal pH, stimulate ruminal fermentation, increase feed intake and milk yields (MY) and reduce risks associated with abrupt dietary changes (Yoon and Stern, 1995; Denev et al., 2007).

Studies regarding use of *Saccharomyces cerevisiae* yeast based supplements date back to the 1950s (Newbold, 1996), and continue to be undertaken today. Positive effects of adding yeast culture to ruminant diets have been reported for growing cattle and lactating dairy cows (Dann et al., 2000; Desnoyers et al., 2009; Yuan et al., 2015). Recently, confirmation of positive effects of using yeast

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culture in lactating cow diets in the transition period and early lactation has been published (Schingoethe et al., 2004; Yuan et al., 2015; Zaworski et al., 2014).

There are only a limited number of studies on the effect of using live yeast supplementation on lactating buffaloes and only 4 relevant references were found between 2008 and 2013 (Campanile et al., 2008; Gaafar et al., 2009; Khattab et al., 2010). The fourth, and most recent publication, was by Degirmencioglu et al. (2013) about effect of *S. cerevisiae* supplementation in lactating Anatolian water buffaloes. This research reported increased dry matter intake (DMI) and total MY and fat corrected milk yield (FCM) in dairy cows after daily *S. cerevisiae* supplementation with 30 g per 500 kg BW. Furthermore, yeast supplementation can affect blood metabolites. A decreased urea N in blood plasma in dairy cows and an increased albumin in ewes were reported after yeast supplementation (Bruno et al., 2009; Helal and Abdel-Rahman, 2010). The responses to yeast culture supplementation, documented in published research, varies and may be due to differences such as the yeast type and strain, mode of action and level of application, as well as the animal type, diet, energy level, parity, lactation stage, and level of productivity. These differences make it difficult to compare published results and predict the usefulness of yeast supplementation for Egyptian buffaloes. Therefore, this study investigated effects of yeast supplementation to Egyptian buffaloes. Specifically, the following hypotheses were tested:

1) Yeast culture supplementation effects are similar in primiparous (PP) and multiparous (MP) lactating buffaloes.
2) Yeast culture supplementation effects are similar in early and mid-lactation buffaloes.
3) Yeast culture supplementation promotes energy and protein conversion efficiency.

2. Materials and methods

2.1. Animals, diets, feeding and experimental design

This study was conducted at the Experimental and Research Station, Shalkan, Faculty of Agriculture, Ain Shams University, Egypt and the laboratories of the Dairy Science Department, National Research Centre, Dokki, Giza, Egypt. Yea-Sacc^{1026} was used as a feed supplement. Yea-Sacc^{1026} is a yeast culture based on a proprietary strain of *S. cerevisiae*. The commercial product has a minimum concentration of 1×10^9 cfu/g, (Alltech Inc, Lexington, KY, USA).

Twenty-four lactating Egyptian buffaloes (8 PP and 16 MP) with a live weight of 520.4 ± 10.47 kg were randomly assigned to 2 groups of 12 buffaloes each, according to parity (4 PP and 8 MP). The animals were fed the experimental feed ration from approximately 15 days before parturition in order to adapt to the feed. All sampling started 15 days after parturition, approximately 30 days after introduction to the feed. The lactation trial lasted 180 days. The animals were housed in an insulated barn and fed individually. The animals were fed a total mixed ration (TMR, Table 1) without or with 10 g Yea-Sacc^{1026} per cow per day as the control and treatment group, respectively. The ration ingredients and chemical composition of the TMR are presented in Table 1. The ration contained 75% to 76% roughage (R) and 24% to 25% concentrate (C) on a fresh matter basis. The total ration was formulated to keep the neutral detergent fiber (NDF), non-fiber carbohydrate (NFC) and net energy for lactation (NE_L) levels according to NRC (2001) recommendations. The rations were formulated to provide the necessary energy and protein requirements according to Paul et al. (2002). Rice straw was available *ad libitum* in addition to the TMR. The ration was offered twice daily at 07:00 and 18:00 and the animals had continuous access to fresh water. Ten grams of the yeast supplement powder were added on top of a quarter of the morning TMR feed. The rest of the TMR and rice straw was given to the buffalo cows only after this feed and the live yeast culture was completely consumed. All experimental animals were cared for according to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). Data from 2 animals (1 PP and 1 MP) were eliminated from the control group due to illness not related to the experiment.

2.2. Sampling

The daily offered feeds (TMR and rice straw) and subsequent orts were recorded for each animal in order to calculate feed intake. Body weight, daily MY, milk fat and milk protein content were registered once every 15 days until 90 days post-partum and thereafter every 30 days until 180 days. The buffaloes were milked twice daily at 04:00 and 17:00 and MY of each buffalo was recorded by the DeLaval milk manager software attached to the milk set. The animals were weighed and milk samples taken at 15-day intervals (days 15, 30, 45, 60, and 75) and thereafter at monthly intervals (days 90, 120, 150 and 180). On the designated sample day, milk from the morning and evening milking of each buffalo cow was pooled and stored at 4 °C for subsequent analyses. The samples were pooled in quantities relative to the total amount of milk produced by the individual buffalo at the respective milking. In this way, one composite milk sample per animal per sampling day was analyzed. Milk samples were analyzed for total solids, fat, true protein and lactose by a Bentley 150 infrared milk analyzer (Bentley Instruments, Chaska, MN, USA). The Bentley instruments company calibrated the machine specifically for Egyptian buffalo milk.

Blood samples were taken from 3 PP and 4 MP control animals and 4 of each PP and MP yeast supplemented buffaloes at 15, 30, 60, 90, 120, 150 and 180 days in milk (DIM). A sample of 10 mL blood was drawn from the jugular vein of each animal. The blood samples were collected directly into clean, dry glass culture tubes at 3 h post morning feeding. The blood samples were centrifuged 2 h after collection at 1,430 × *g* for 15 min to collect serum. The serum was stored at −20 °C in clean, dry glass vials until subsequent analyses. The serum samples were analyzed using commercial kits (SPINREACT, A. A. Ctra. Santa Coloma, Girona, Spain). Total protein, albumin, urea, and creatinine concentrations were used as an indication of kidney function, while alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were used as an indication of liver damage and total lipids as an indication of fat mobilization. Globulin concentration was calculated by subtraction of total serum protein and serum albumin. The albumin/globulin (A/G) ratio was calculated by dividing the value of albumin by the value of globulin in serum. These values are used to assess growth and general health.

Digestibility sampling was undertaken twice during the lactation trial. Fecal samples (approximately 150 g) were collected at 08:00 and 16:00 from the rectum for 3 consecutive days from days 45 to 47 and from days 90 to 92 and pooled by buffalo. These 2 sampling periods were considered to be representative of early and mid-lactation, respectively. A solution of 10% H_2SO_4 and formalin was added to the sample (Khattab et al., 2012). The samples were subsequently dried at 55 °C for 48 h, ground in Wiley mill to pass a 1 mm sieve, and thereafter subjected to chemical analysis. The acid insoluble ash (AIA) technique (Van Keulen and Young, 1977) was used as an internal marker for nutrient digestibility calculation as suggested by Sales and Janssens (2003).

2.3. Chemical analysis and calculations

Samples of the TMR and rice straw were collected, pooled weekly, completely dried at 55 °C and ground to pass a 1 mm screen

Table 1
Ingredients and chemical composition of the ration fed to lactating buffalo cows (DM basis).

Ingredient	Ration content, g/kg	Chemical composition of the ration	Content, g/kg DM
Berseem	730	Organic matter	883
Rice straw	29.2	Crude protein	126
Yellow corn	134	Ether extract	32.3
Soybean meal	50.6	Crude fiber	165
Wheat bran	36.1	Non fiber carbohydrates	345
Sunflower meal	14.5	Neutral detergent fiber	378
$CaCO_3$	2.41	Acid detergent fiber	229
Minerals and vitamins[1]	2.41	NE_L[2], Mcal/kg DM	1.75
NaCl	0.69		

[1] Ca, 141 g/kg; P, 27 g/kg; Mg, 65 g/kg; S, 14 g/kg; Na, 120 g/kg; K, 6 g/kg; Fe, 944 mg/kg; Zn, 1,613 mg/kg; Cu, 484 mg/kg; Mn, 1,748 mg/kg; I, 58 mg/kg; Co, 51 mg/kg; Se, 13 mg/kg; vitamin A, 248,000 IU/kg; vitamin D_3, 74,000 IU/kg; vitamin E, 1,656 IU/kg.
[2] NE_L = net energy for lactation, calculated using the equation for NE_L (NRC, 2001).

in a Wiley mill before analyses. Feed and feces samples were analyzed in triplicate for dry matter (DM), ash, crude fiber (CF), crude protein (CP) (Nitrogen × 6.25) and ether extract (EE) contents according to AOAC (2000). Neutral detergent fiber and acid detergent fiber (ADF) contents were analyzed according to Van Soest et al. (1991) at the Animal Nutrition Laboratory at the Animal Production Department, Ain Shams University in Egypt. Non-fiber carbohydrate was calculated according to the following formula (NRC, 2001):

$$\text{NFC}\ (\%) = 100 - (\%\text{NDF} + \%\text{CP} + \%\text{fat} + \%\text{ash}).$$

The digestibility coefficient of a given nutrient was calculated according to the following formula (Van Keulen and Young, 1977):

$$\text{Digestibility} = 100 - \left(100 \times \frac{\%\text{indicator in feed}}{\%\text{indicator in feces}} \times \frac{\%\text{indicator in feces}}{\%\text{nutrient in feed}}\right)$$

Average energy and protein conversion efficiency was calculated for each buffalo, based on the total intake and total yields in each period. Energy corrected milk was calculated according to equation of Tyrrell and Reid (1965) as follows:

$$\text{ECM} = 0.327 \times \text{Milk yield (kg)} + 12.95 \times \text{Fat yield (kg)} + 7.20 \times \text{Protein (kg)}.$$

Milk energy was calculated as the quantity of ECM produced (kg) × 0.692 (Mcal/kg) (Tyrrell and Reid, 1965). Intake of NE_L was calculated as DMI of concentrate and forages multiplied by the estimated NE_L contents of the forage (NRC, 2001). Feed efficiency is expressed as the amounts (kg) of MY and ECM per kg DMI. Energy conversion efficiency is the ratio of milk energy output to NE_L consumed. Nitrogen conversion efficiency is the ratio of milk nitrogen yield to nitrogen intake.

2.4. Statistical analysis

Initial body weight, weight gains from 15 to 180 days, total MY, total ECM yield, daily ECM yield from parturition (0 kg milk) until day of maximum yield and daily ECM yield from day of maximum yield until the end of experiment, feed conversion and energy and nitrogen efficiency results were analyzed using the following linear model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ij},$$

where Y_{ij} is the value of the ijth observation, μ is the overall mean, α is the fixed effect of treatment ($_i$ = yeast or control); β is the fixed effect of parity ($_j$ = PP or MP), $\alpha\beta$ the interaction between parity and treatment, and ε is the residual error.

Apparent digestibility results were analyzed with the addition of the fixed effect of stage of lactation (pooled days of sampling) as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \varepsilon_{ijk},$$

where Y_{ijk} is the value of the ijkth observation, μ is the overall mean, α is the fixed effect of treatment ($_i$ = yeast or control), β is the fixed effect of parity ($_j$ = PP or MP), γ is the fixed effect of stage of lactation ($_k$ = days 45 to 47 or days 90 to 92). The interactions between treatment and parity, treatment and day, and parity and day are $\alpha\beta$, $\alpha\gamma$, and $\beta\gamma$, and $\alpha\beta\gamma$ is the three-way interaction between parity, treatment and day. The residual error is ε.

Repeated blood measure results were plotted as longitudinal data over time. The data were analyzed with the following mixed random model with correlated effects for day of sampling for each animal.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_k + \left(\gamma\delta_{ijk}\right) + \varepsilon_{ijk},$$

where Y_{ijk} is the value of the ijkth observation, μ is the overall mean, α the fixed effect of treatment ($_i$ = yeast or control), β the fixed effect of parity ($_j$ = PP or MP), $\alpha\beta$ their interaction, γ the day of the sampling ($_k$ = days 15, 30, 60, 90, 120, 150 and 180), and $\gamma\delta$ is the random effect of date of sampling of each animal. The residual error is ε.

All models were reduced stepwise to only include significant variables for all statistical analyses. The error terms and the random-effects variables are assumed to have a normal distribution with mean zero and variances $\sigma^2_{\varepsilon R}$ (residual error). Model validation was carried out using visual inspection of residuals and Cook's distances. Results were considered significantly different when $P < 0.05$ and a tendency is concluded when $0.05 < P < 0.10$. Statistical analyses were performed using the software R (R Development Core Team, 2016), the lmer package for linear mixed models (Bates et al., 2014) and the nlme package for linear models with repeated measurements (Pinheiro et al., 2016).

3. Results and discussion

3.1. Digestibility

Apparent digestibility of OM, DM and CP were significantly greater at mid-lactation sampling (days 90 to 92) than at early lactation (days 45 to 47; Table 2), regardless of treatment and parity. The apparent digestibility of DM and OM were significantly different for both treatment (yeast > control) and stage of lactation (days 90 to 92 > days 45 to 47) (Table 2). No significant differences for parity or any interactions between stage lactation, parity or

treatment were found for DM, OM or CP apparent digestibly coefficients. However, a significant ($P = 0.033$) three-way interaction between stage of lactation, treatment and parity for CF digestibility was observed. Therefore, individual analyses for early and mid-lactation were undertaken. The average DM, OM, CP and CF digestibility coefficients for yeast supplemented and control PP and MP buffaloes are shown in Table 2, and significant main effects for each lactation stage are indicated. There were no significant interactions between parity and treatment at early or mid-lactation and neither treatment nor parity significantly affected the results of CF digestibility at early lactation. A significant difference for parity (MP > PP; $P = 0.049$) and treatment (treated > control, $P = 0.012$) was seen at mid-lactation.

Using a composite sample from days 45 to 47 to denote early lactation and from days 90 to 92 to denote mid-lactation was an estimate used when planning digestibility sampling. Actual time to peak yield was only less than 45 days in the primiparous yeast treated group (average of 41 days). This is in alignment with the results by Chaudhry et al. (2000) who found that peak yield occurred 48 days post-partum in Nili-Ravi buffaloes in Pakistan. However, no comparable research was found for Egyptian buffalo cows, and more research is needed to investigate yeast supplementation effects on lactation curve kinetics.

Crude fiber digestibility differed according to parity only at the mid-lactation sampling. No differences were found due to parity or treatment at early lactation, but the digestibility for second parity buffaloes fed the yeast supplement was significantly greater than the control group during mid-lactation. The differences found for both PP and MP buffalo cows during mid-, but not early lactation, may have been due to the following: 1) increased feed retention time after peak lactation; 2) the need for a longer adaptation period than suggested by the producers; 3) lack of detection of a real effect of yeast supplementation during early lactation; and/or 4) that no real difference exists at early lactation, regardless of retention time.

The response in CF digestibility during mid-lactation as compared to early lactation is most likely due to a longer feed retention time after peak yield, as has been shown in dairy cattle (Ishler and Varga, 2001). A longer adaptation period may not be necessary given the recommendation from the additive producers (AlltechInc, Lexington, KY, USA) and positive results from other studies that used 4 weeks adaptation (de Ondarza et al., 2010). However, more research is needed to differentiate these possible causes.

The significant increased CF digestibility during mid-lactation by parity (64.0% vs. 61.2%, $P = 0.049$) for MP vs. PP is most likely due to reduced feed retention time before peak lactation and longer feed retention time after peak yield in the older buffaloes, larger DMI capacity and greater DMI and greater MY. This would be, regardless of treatment, expected to result in a relatively greater apparent digestibility. The significant response of CF digestibility at mid-lactation to yeast treatment (65.9% vs. 59.3%, $P = 0.012$) may indicate that fiber digestion is particularly sensitive to yeast supplementation. Yeast culture has been shown to increase ruminal pH in dairy cows and fattening goats (Marden et al., 2008; Özsoy et al., 2013). Increased pH has, in turn, shown to increase the number of cellulolytic bacteria (Newbold et al., 1996) and number of mesophilic bacteria (Özsoy et al., 2013). Therefore, using a live yeast supplementation could be beneficial in order to stimulate fiber digestion. Increased OM, CP, CF, NDF and ADF digestibility has also been observed with MP buffaloes using the same product and dose as the present research (Campanile et al., 2008; Khattab et al., 2010; Azzaz et al., 2015). These publications report increased digestibility results using different yeast products and different doses in MP buffaloes. Similar positive effects on digestibility were found when using Baker's yeast in both 60:40 and 40:60 roughage to concentrate rations for MP buffaloes (Gaafar et al., 2009). Bitencourt et al. (2011) found a numerically, non-significant increase for DM and OM digestibility with dietary yeast supplementation, but NDF digestibility only tended to be better for the yeast supplemented dairy cows (48.1% vs. 43.2%, for yeast treated and control respectively, $P = 0.08$). In contrast to the results from this study and other published results, Bagheri et al. (2009) found the yeast supplementation in dairy cows increased DM and CP digestibility but did not affect NDF digestibility compared to the control animals. The observed variation of responses between studies may be partially related to factors such as differences of method of fiber determination, yeast dose, yeast sources, amount of viable yeast in products, animal parity, forage type, ration composition, lactation stage and seasonal effects.

3.2. Blood metabolites

Use of a model with correlated random effects of day was only necessary for A/G ratio and creatinine, and for other metabolites, the model was reduced. No significant interactions were found and therefore only the significance of the main effects is indicated in Table 3. The albumin and A/G ratio significantly differed ($P \leq 0.039$) with respect to parity. All values of the parameters in Table 3 are within in the reference intervals for healthy buffaloes (Abd Ellah et al., 2014). An effect of yeast treatment was observed only in the measure of total lipids (1.42 vs. 1.11 mg/dL for control vs. yeast treated buffalo cows, $P = 0.029$).

Blood metabolites are frequently used to scan for metabolic health status in dairy herds (Ametaj et al., 2009). In the present

Table 2
Effect of yeast culture supplementation on the apparent digestibility (%, means ± SEM) of the ration dry matter (DM), organic matter (OM), crude protein (CP) and crude fiber (CF) for lactating buffalo cows at early (days 45 to 47) and mid-lactation (days 90 to 92).

Item	Lactation stage	Primiparous		Multiparous		Main effects[1] (*P*-value)		
		Control	Yeast	Control	Yeast	T[2]	P[3]	Lactation stage
Number of animals		3	4	7	8			
DM	Early	57.6 ± 6.52	58.4 ± 3.00	57.0 ± 2.18	60.8 ± 1.63	NS	NS	***
	Mid	60.7 ± 1.05	69.8 ± 0.49	64.2 ± 1.35	66.5 ± 1.07	*	NS	
OM	Early	64.7 ± 4.41	63.2 ± 2.44	62.3 ± 2.38	65.7 ± 1.63	NS	NS	***
	Mid	63.7 ± 0.95	72.9 ± 0.72	67.4 ± 1.49	70.4 ± 1.05	*	NS	
CP	Early	71.9 ± 7.90	63.5 ± 4.08	63.5 ± 2.38	64.7 ± 2.36	NS	NS	***
	Mid	68.4 ± 0.64	73.5 ± 2.68	70.8 ± 4.45	72.2 ± 1.98	NS	NS	
CF	Early	56.6 ± 3.72	52.7 ± 3.58	55.4 ± 2.24	58.7 ± 2.19	NS	NS	***
	Mid	55.4 ± 2.39	66.9 ± 2.12	63.2 ± 2.47	64.9 ± 1.75	*	*	

***: $P < 0.001$; *: $0.01 < P < 0.05$; NS: $P > 0.05$.
[1] Only significance of main effects indicated for each lactation stage, as no significant two-way interactions were detected.
[2] T: effect of treatment.
[3] P: effect of parity.

Table 3
Effect of yeast culture supplementation on selected blood metabolites in lactating buffalo cows (means ± SEM).

Item	Primiparous		Multiparous		Main effects[1] (*P*-value)		
	Control	Yeast	Control	Yeast	T[2]	P[3]	Day
Number of animals	3	4	7	8			
Total protein, g/dL	5.99 ± 0.092	6.08 ± 0.165	6.05 ± 0.132	6.18 ± 0.106	NS	NS	NS
Albumin, g/dL	3.49 ± 0.340	3.23 ± 0.515	3.90 ± 0.264	3.90 ± 0.335	NS	*	NS
Globulin, g/dL	2.49 ± 0.363	2.84 ± 0.530	2.15 ± 0.215	2.27 ± 0.304	NS	NS	NS
A/G ratio	1.53 ± 0.346	1.44 ± 0.460	2.03 ± 0.336	2.34 ± 0.650	NS	*	*
Creatinine, mg/dL	0.80 ± 0.167	0.83 ± 0.175	0.86 ± 0.158	1.01 ± 0.183	NS	NS	*
Urea, mg/dl	49.9 ± 14.26	51.2 ± 17.80	55.1 ± 10.469	49.4 ± 8.379	NS	NS	NS
ALT, U/L	38.9 ± 9.12	54.5 ± 9.95	39.4 ± 7.89	48.7 ± 8.16	NS	NS	NS
AST, U/L	68.0 ± 3.14	65.7 ± 2.71	66.0 ± 2.29	65.3 ± 2.90	NS	NS	NS
Total lipids, mg/dL	1.56 ± 0.288	1.18 ± 0.330	1.31 ± 0.192	1.02 ± 0.169	*	NS	NS

A/G ratio = albumin/globulin ratio; ALT = alanine aminotransferase; AST = aspartate aminotransferase.
*: $0.01 < P \leq 0.05$; NS: $P > 0.05$.
[1] Only significance of main effects indicated, as no significant interactions were found.
[2] T: effect of treatment.
[3] P: effect of parity.

study, values for total protein, albumin, globulin, A/G ratio, creatinine, urea, enzyme activities of ALT and AST were not altered by yeast culture supplementation (Table 3). Research with lactating ewes (Helal and Abdel-Rahman, 2010; Baiomy, 2011), dairy cows (Bagheri et al., 2009), and goats (Özsoy et al., 2013) also found that blood metabolites such as total protein, globulin and urea, AST and ALT were not affected by supplementation with yeast culture. However, Helal and Abdel-Rahman (2010) reported that blood plasma serum albumin significantly increased in lactating ewes fed yeast supplement but only noted that the values were within normal range. The concentration of urea N in plasma was reduced after feeding yeast culture to dairy cows (Bruno et al., 2009). No significant difference was found for urea between yeast treated or control buffalo cows in the present study.

Bruno et al. (2009) suggested that reduced urea N in the plasma of yeast treated cows indicates improved protein utilization. Milk protein production increased significantly in the present study with yeast supplementation. However, this effect was seen only in MP buffalo cows that ate more DMI, produced more milk and had a numeric, but not significantly, greater nitrogen conversion efficiency than the controls. The significant decrease of blood lipids for yeast-supplemented animals, regardless of parity, can indicate decreased fat mobilization in both MP and PP buffalo cows. Several studies (Bruno et al., 2009; Baiomy, 2011; Helal and Abdel-Rahman, 2010; Kalmus et al., 2009) have reported the effect of yeast supplementation on fat metabolism derivatives (non-esterified fatty acids [NEFA], beta-hydroxybutyric acid [BHBA], cholesterol, and triglycerides). These studies report inconsistent results that fluctuated between increased and decreased levels of metabolites. The difference in these results most likely due to differing parities, stage of lactation, dose, ration composition, and animal species.

3.3. Body weight, feed intake, milk yield and composition

As expected, MP buffalo cows weighed significantly more, ate significantly more DM, had significantly greater MY, ECM, milk fat and protein, and had a significantly better feed conversion, energy and nitrogen conversion efficiency than PP buffalo cows ($P < 0.05$). No significant interaction was found between parity and treatment. Therefore, levels of significance are only presented for treatment within parity in Table 4. The body weight at day 15 was significantly different by parity ($P = 0.009$), not significantly different between the PP groups ($P = 0.358$) and tended ($P = 0.057$) to be significantly different for the MP treatment groups. The average of the yeast treated group of PP animals was 21 kg less than the control group at day 15, but this was not significant due to a large variation. There were no differences by parity or treatment for total weight gained during the experiment.

Body weight in dairy cows tends to decrease in early lactation (McDonald et al., 2002). Early lactation weight loss represents an imbalance between energy intake and the required energy for milk production. Primiparous animals, when compared to MP animals, need to use relatively more of their consumed energy for growth instead of milk production. The lack of significant difference in weight gains for both MP and PP animals may indicate that yeast supplementation does not affect total weight gain. Alternately, the large variation seen in the present research could indicate a need for more research. However, weight gains are used as an indicator of energy partitioning, and should be evaluated together with intake and milk production.

The effect of yeast supplementation on weight gains varies in published research. Degirmencioglu et al. (2013) did not find significant weight gain when MP buffaloes were fed a supplement of 30 g *S. cerevisiae* to alfalfa and concentrates for 28 days. In contrast, Khattab et al. (2010) reported that MP buffaloes given 10 g dry yeast in the diet had a significantly greater weight gain than the controls. The dose used by Khattab et al. (2010) was the same and animal weights were similar to those used in the present research. Lehloenya et al. (2008) also found that yeast supplemented MP cows gained more compared to control cows during early and mid-lactation. Özsoy et al. (2013) added another live yeast culture (RumiSacc 1.4×10^8 cfu/g) in the concentrate portion of the ration to fattening male goat kids at 4 levels (0, 1.5%, 3.0% and 4.5% of diet as fed) for 70 days. The maximum dose investigated in this research (4.5%) increased total weight gain (+15.5%, $P = 0.010$) compared to the control group. The lack of significant weight change for PP buffalo cows found in present study is consistent with 2 of the 3 published research results found about yeast supplementation in PP cows (Lehloenya et al., 2008; Moallem et al., 2009). Szucs et al. (2013) investigated yeast supplementation in PP cows, but did not record weight changes. The kinetics of weight gain after peak production are affected by the diet nutritional density, duration of supplementation, level of MY, shape of lactation curve and degree of body weight mobilization. These factors will affect the subsequent weight gains.

The yeast treated MP buffaloes consumed significantly more DM and CP per day (8.30% and 8.24%, respectively), produced more MY, ECM, milk fat and milk protein than their controls. Yeast supplemented MP buffalo cows produced 362 kg ECM, 17 kg fat and 17 kg protein more than MP control buffalo cows during 180 days. Dry

Table 4
Effect of yeast culture supplementation on body weight, feed intake, milk yield and feed conversion and efficiency in lactating buffalo cows (means ± SEM).

Item	Primiparous		*P*-value	Multiparous		*P*-value[1]
	Control	Yeast		Control	Yeast	
Number of animals	3	4		7	8	
Body weight, kg						
Weight at day 15	450 ± 17.9	429 ± 12.5	NS	516 ± 15.5	553 ± 9.5	0.057
Weight gain (15 to 180 days)	25.7 ± 18.45	27.0 ± 9.41	NS	30.1 ± 7.00	26.7 ± 10.96	NS
Feed intake						
DMI, kg/d	10.46 ± 0.866	9.96 ± 0.235	NS	10.72 ± 0.275	11.6 ± 0.268	0.041
CP intake, g/d	1,313.2 ± 111.42	1,250.5 ± 29.50	NS	1,346.4 ± 34.77	1,457.4 ± 33.94	0.030
Yield and composition						
Milk yield, kg/d	6.8 ± 1.03	6.4 ± 0.35	NS	7.2 ± 0.34	8.4 ± 0.35	0.027
ECM, kg/d	10.5 ± 1.61	10.1 ± 0.50	NS	11.2 ± 0.52	13.2 ± 0.56	0.030
Milk fat, g/d	475.5 ± 75	462.6 ± 240	NS	508.3 ± 26.45	603.7 ± 29.34	0.033
Milk protein, g/d	300.2 ± 29.44	277.7 ± 15.50	NS	316.2 ± 15.87	367.2 ± 15.20	0.022
Feed conversion efficiency						
Milk yield/DMI, kg/kg	0.64 ± 0.051	0.65 ± 0.030	NS	0.68 ± 0.022	0.73 ± 0.017	NS
ECM/DMI, kg/kg	0.99 ± 0.080	1.02 ± 0.050	NS	1.05 ± 0.034	1.13 ± 0.028	NS
Energy efficiency, %	42 ± 6.3	43 ± 3.5	NS	44 ± 2.2	47 ± 2.4	NS
Nitrogen efficiency, %	22 ± 1.7	22 ± 1.0	NS	23 ± 0.7	24 ± 0.3	NS

DMI = dry matter intake; CP = crude protein; ECM = energy corrected milk.
NS: $P > 0.05$.
[1] Only significance of treatment within parity are shown, as no significant interactions were found.

matter intake, CP intake, MY, ECM and milk fat and protein yield did not differ significantly for yeast supplemented PP buffalo cows compared to their controls.

The DMI results are in agreement with those reported by Degirmencioglu et al. (2013), who observed that yeast supplemented MP buffaloes consumed 5.4% more DM than their controls, and results from Yuan et al. (2015), who observed a 4.6% increase in DMI in dairy cows given yeast supplementation. Increasing DMI in MP buffaloes was reported with differing yeast supplementation levels and differing concentrate to roughage ratios (Gaafar et al., 2009). An increased DMI was reported for lactating ewes fed yeast supplements (Helal and Abdel-Rahman, 2010). Increased DMI with yeast supplementation was seen during the transition period as well as early, mid-, and late lactation in cows (Ramsing et al., 2009). Finally, Dann et al. (2000) also found increased DMI before calving in yeast supplemented Holstein cows with an increased DMI persisting until 140 days postpartum.

Three studies have reported no effect of yeast supplementation on DMI, even when using the same strain of yeast (Campanile et al., 2008; Khattab et al., 2010; Kumar et al., 2011). The present data agree with the studies that found a stimulating effect of yeast on DMI and suggests that yeast products, despite type of animal, lactation stage, dose, or ration composition may improve feed intake. This may be due to a modified rumen metabolism and increased nutrient digestibility, in particular fiber.

Increased feed intake and improved fiber digestibility can lead to increased milk production or increased weight gain. Supplementation of live yeast improved MY, milk fat and protein yields significantly in MP buffaloes (Table 4). Yeast supplementation has been reported to increase MY and ECM yields by 6.7% and 10%, respectively (Campanile et al., 2008). Degirmencioglu et al. (2013) found a 14% MY increase and a 24% increase in FCM yield, but neither of these studies found significant effects of yeast supplementation on milk fat and protein concentration in buffalo cows. Yeast supplementation also increased daily MY (29.4 vs. 28.5 kg, $P = 0.11$) and protein (0.939 vs. 0.908 kg, $P = 0.05$), but did not affect milk fat content in dairy cows (Bitencourt et al., 2011). The response of yeast on the production and milk composition of lactating buffaloes is most likely the result of increased feed intake and improved digestibility of nutrients.

The results from this and other research are in contrast to those obtained by Szucs et al. (2013) Ramírez et al. (2007) and Bagheri et al. (2009). Szucs et al. (2013) did not find differences between the daily MY, milk composition and yield of protein or fat between yeast supplemented and control MP cows during first 100 days postpartum, but found that PP cows fed a yeast supplement had a better yield compared to control group. Ramírez et al. (2007) found no difference in milk yield and composition after feeding *S. cerevisiae* yeast culture to water buffalos in Colombia and Bagheri et al. (2009) concluded that yeast supplementation did not affect high yielding Holstein dairy cows in early lactation.

3.4. Feed conversion, energy and nitrogen conversion efficiency

Feed conversion, energy and nitrogen conversion efficiency were numerically better for the yeast treated buffaloes, but did not differ significantly ($P > 0.05$) for either PP or MP yeast fed buffaloes compared to the respective controls. One of the key reported benefits of yeast supplementation is improved feed efficiency (Schingoethe et al., 2004). The present study showed a slight improvement ($P > 0.05$) of feed conversion, energy and nitrogen conversion efficiency within each parity for yeast supplemented buffalo cows. Yuan et al. (2015) also found that yeast supplementation tended to improve energy conversion efficiency, independent of dose. They suggested that the response in energy efficiency could be due to one or any combination of the following: 1) enhanced energy digestibility; 2) decreased maintenance energy requirements; or 3) improved efficiency of net energy use for milk synthesis. Desnoyers et al. (2009) suggested that yeast supplementation increases OM digestibility in ruminants, thereby increasing energy harvest from the diet. These theories are consistent with the results from the present study. No published work was found comparing the effect of yeast supplementation on feed conversion, or nutrient conversion efficiency in PP versus MP buffalo cows.

3.5. Rate of production before and after peak milk yield

Despite the differences of total milk and ECM yields, no significant differences were found in the daily milk production before (during early lactation) or after peak MY (during mid-lactation) according to treatment or parity (Table 5). The overall increase in daily milk production from parturition (0 L milk) until peak production for control and treated animals was 311.9 (SEM = 17.1) and

Table 5
Effect of yeast culture supplementation on average daily production of energy corrected milk before and after peak yield in lactating buffalo cows (means ± SEM).

Item	Primiparous		Multiparous	
	Control	Yeast	Control	Yeast
Number of animals	3	4	7	8
Average days to peak yield (min. to max.)	50 (15 to 75)	41 (30 to 60)	54 (30 to 75)	51 (30 to 60)
Peak daily yield, L	12.3 ± 1.59	11.8 ± 0.78	13.1 ± 0.66	14.8 ± 0.85
Average daily increase from parturition until peak yield, mL	421.0 ± 198.68	301.5 ± 29.67	265.1 ± 34.12	301.7 ± 19.47
180-day yield, L	9.6 ± 1.36	9.1 ± 0.39	9.5 ± 0.51	11.6 ± 0.62
Average daily decrease from peak yield until 180-day yield, mL	−22.4 ± 12.15	−20.0 ± 5.57	−29.6 ± 4.67	−26.4 ± 8.08

301.6 (SEM = 68.1) mL/day, respectively. After peak yield the overall average yield decreased by 27.4 mL/day (SEM = 5.0) for control and slightly less (24.3 mL/day; SEM = 6.3) for yeast treated buffaloes. The variation, in particular for the PP control buffalo cows, was very large with a single cow producing 901 mL milk per day from parturition to peak yield at 15 days in milk. Primiparous cow yields, until peak yield, can show large variation (Kessler et al., 2014) and this could be expected in buffalo cows.

A numerical, but not significant, increase in milk production before peak yield and more persistent milk production after peak yield occurred for yeast fed MP buffalo cows (Table 5). The numerical, but not significant, more persistent curve was also evident for yeast supplemented PP buffalo cows. This resulted in significant ($P = 0.03$) increased MY, ECM, milk protein and fat yield for yeast supplemented MP buffalo cows. The slower decrease of milk production after peak yield for yeast treated PP buffalo cows suggests that yeast may also have a positive effect after peak yield in younger animals that have not achieved mature weight.

Increased feed intake and increased digestibility of fiber, with no detected difference in weight gains as well as a decreased fat mobilization resulted in significantly greater MY and ECM yield for yeast supplemented MP buffalo cows. The same significant effects were not seen in yeast treated PP buffaloes.

4. Conclusions

Yeast supplementation affects MP and PP buffalo cows differently. Multiparous cows respond to yeast supplementation through increases in intake, DM, OM, CF digestibility, MY, milk fat and protein yields. Both MP and PP buffalo cows appear to respond to yeast supplementation by reduced fat mobilization. Supplementing buffaloes with yeast culture may increase milk production before peak yield during early lactation and result in a more persistent milk production after peak yield during mid-lactation. Feed conversion, energy and nitrogen conversion efficiency may be increased with the use of yeast supplementation.

Conflict of interest

There were no conflicts of interest with this research.

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